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ROY WALDO MINER

GLUTATHIONE CONTROL OF THE SPECIFIC FEEDING REACTIONS OF HYDRA

BY

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The Loomis Laboratory, Greenwich, Conn.

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The fact that hydra feed *exclusively* on living animals was first described by Trembley in a classical memoir published in 1744. Trembley showed that hydra do not chase their prey, but wait passively for small aquatic animals to swim up against one of their tentacles (FIGURE 1). When this happens, the prey is immediately arrested and can only make ineffectual efforts to escape. Shortly thereafter, the capturing tentacle contracts, bringing the prey towards the mouth of the hydra which then opens and swallows it. Trembley noted, furthermore, that the total removal of the tentacles from a hydra still permitted it to swallow injured prey placed against its mouth (Baker, 1952).

Subsequent workers have confirmed and extended Trembley's observations. They have shown that hydra will feed on many different species, including nematode and annelid worms, crustacea, insect larvae, arachnids, and even vertebrates such as tadpoles and newly-hatched fish. Hydra will not feed, however, on dead specimens of these same species (Hyman, 1940).

In 1924, Beutler observed that hydra would ingest small pieces of gelatine or fibrin soaked in crustacean juice. In 1947, Ewer showed that such juice caused hydra to contract their tentacles and open their mouths even when no particulate matter was present. She concluded: "In normal feeding two independent processes are involved, the discharge of the nematocysts (or stinging cells present on the tentacles) and the 'feeding reaction.' The nematocysts are independent effectors, while the feeding reaction is carried out by the neuromuscular system. In this reaction the tentacles writhe and twist towards the mouth, while the mouth itself opens widely." During some recent experiments on regeneration (1953), Park made the curious observation that hydra open their mouths in the presence of glutathione (personal communication).

It has often been thought that hydra feed exclusively on living prey because only living prey is motile and hence able to contact the passively outstretched tentacles of hydra (Whitney, 1907). Although motility is undoubtedly an important factor, it does not explain Beutler's observation that hydra will not swallow bits of gelatine or fibrin unless they are first soaked in crustacean juice.

The nature of the mechanism by which hydra differentiate between living and dead prey is of considerable interest, for hydra possess neither a brain nor sensory organs such as the eyes of higher animals.

Hadzi (1909) has shown that the nervous system of hydra consists of individual sensory cells connected by a loose network of nerve cells. Without even a rudimentary "brain," therefore, hydra react selectively to living prey. Furthermore, despite their apparently inadequate nervous system, hydra demonstrate considerable coordination in the feeding reaction. For example, they contract whatever tentacle has succeeded in capturing prey and even open their mouths *in anticipation,* before the prey has actually touched the sensory cells around the edges of the mouth (Buchsbaum, 1948).

The present paper reports the results of a detailed study of the "feeding reaction" of hydra. It demonstrates that this "feeding reaction" is controlled chemically by a "feeding hormone" and not by the neuromuscular system. The chemical mediator involved is specifically identified as reduced glutathione.

Experimental

All of the following experiments were made on *Hydra littoralis* grown in stock culture by a method that has been described previously (Loomis, 1953, 1954).

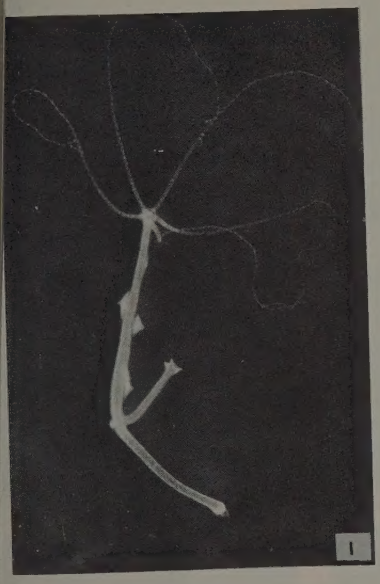
(1) *Preliminary Experiments Indicating the Existence of a Labile "Feeding Hormone"*

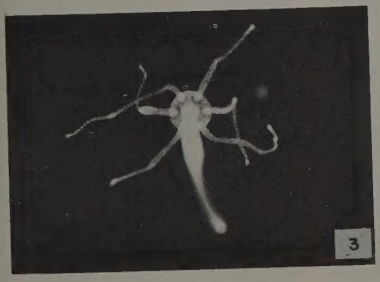
When this problem was first investigated in this laboratory, various attempts were made to feed hydra on dead* material. All of these attempts failed. It was found, for example, that even though hydra feed in nature on the water-flea *Daphnia,* they will not ingest dead daphnia held with a fine pair of forceps and agitated vigorously among their tentacles in simulation of the movements of living prey. Hydra were observed to catch and hold dead daphnia presented to them in this fashion, but no further "feeding reaction" was observed to take place. Instead of being swallowed, the dead daphnia simply remained attached to the tentacles until they were released consequent to the eventual extrusion of the nematocysts that held them.

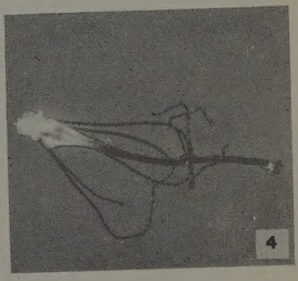
In contrast to the above results, it was found that dead daphnia were acceptable to hydra if first moistened with the juice of a freshly crushed daphnia, in confirmation of the observations of Beutler.

Once the significance of this observation was appreciated, it became possible to investigate the chemical nature of the active principle in-

*Only animals that have been dead for several hours should be used in such experiments. Care must be taken not to contaminate the nonliving particles with even a trace of fresh tissue juice obtained from living animals.







(All photographs are of living specimens,
taken by open flash technique × 15)

FIGURE 1. Normal sexually mature male *Hydra littoralis*. Note extended tentacles and tightly closed mouth.

FIGURE 2. Upper hydra partially inverted. Lower hydra has opened its mouth and has swallowed several of its own tentacles. Both hydra suspended in 10^{-5}M GSH.

FIGURE 3. Hydra attempting to ingest the glass wall of its container under the influence of 10^{-5}M GSH.

FIGURE 4. Partial inversion induced by 10^{-5}M GSH. Note tentacles arising from under the cuff formed by inversion of the mouth.

TABLE 1

STABILITY OF THE ACTIVE PRINCIPLE IN FRESH TISSUE JUICE

Experiment	*Treatment of fresh tissue juice*	*Ability to induce hydra to ingest dead daphnia*
1	Diluted 1/10	+
	1/100	+
	1/1,000	+
	1/10,000	−
2	Unboiled	+
	Boiled	+
3	Diluted 1/1000 and left at 20°C.	
	0 min.	+
	30 min.	+
	60 min.	+
	90 min.	±
	120 min.	0
4	Diluted 1/1000	+
	Treated with 0.1% H_2O_2 for 5 min. and then diluted 1/1000	0

volved. This was carried out by moistening dead daphnia with fresh tissue juice that had been treated in various ways (TABLE 1) and then determining whether or not they would still be ingested by hydra. A convenient source of fresh crustacean juice was found in the brine-shrimp *Artemia*, as large quantities of these crustacea may be easily obtained (Loomis, 1953). When a dense suspension of *Artemia* was homogenized and then centrifuged, the clear supernatant was found to be highly active.

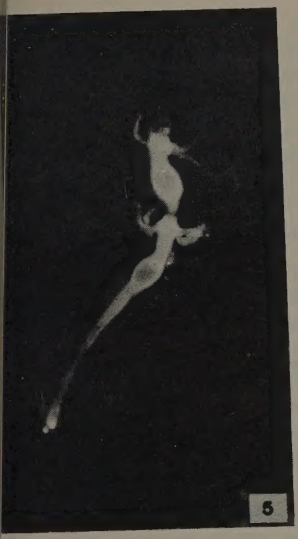
Several conclusions could be drawn from the experiments listed in TABLE 1. First, the active principle was clearly present in considerable quantities, for, it was still active after being diluted one thousand times. Second, the active principle was probably not protein in composition, for boiling did not inactivate the juice. Third, the active principle was

FIGURE 5. Beginning cannibalism among hydra suspended in 10^{-5}M GSH. Note characteristic position of contracted tentacles around mouths.

FIGURE 6. Multiple cannibalism among hydra suspended in 10^{-5}M GSH.

FIGURE 7. Chain cannibalism among hydra suspended in 10^{-5}M GSH.

FIGURE 8. Paralysis of the feeding reaction in hydra suspended in 5×10^{-5}M alloxan. Note extended tentacles and closed mouth despite the presence of numerous *Artemia* on the tentacles. Sexually mature specimen with numerous spermaries.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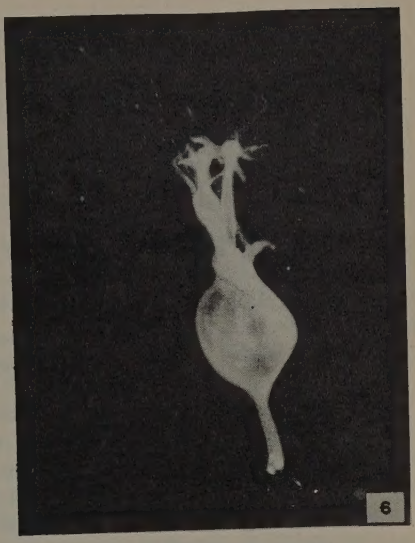
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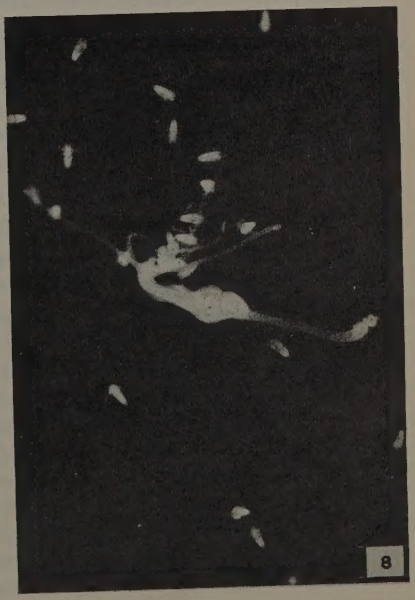
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labile, for it became inactive on standing for two hours at room temperature. The rapid inactivation produced by hydrogen peroxide suggested that this lability was due to a gradual oxidation of the compound to an oxidized form that was biologically inactive. Finally, it appeared that the active principle was widely distributed throughout the animal kingdom, for hydra have been observed to feed on almost all small species of animals (Hyman, 1940).

(2) *Observation of the Hormonal Activity of Glutathione*

Various oxidizable cell constituents were then tried to see whether they could replace fresh tissue juice in the feeding reaction. As cysteine and ascorbic acid were inactive, other compounds were tried including glutathione which, it will be remembered, had been found by Park to cause hydra to open their mouths. It was immediately apparent that glutathione was highly active. Solutions as dilute as 10^{-5}M induced hydra to ingest dead daphnia just as if the daphnia had been moistened with fresh tissue juice. As glutathione in the reduced state (GSH) is known to be (1) present in all animal, plant, and bacterial cells; (2) stable to boiling; and (3) oxidizable to a disulfide form (GSSG) both slowly by air and rapidly by hydrogen peroxide (Barron, 1951), it appeared probable that the active principle present in fresh tissue juice was reduced glutathione. The possibility remained, however, that a trace contaminant in the sample of glutathione tested (Eimer and Amend, C. P. Glutathione) was responsible for its activity. This possibility was eliminated by testing a sample of GSH prepared synthetically by Professor Vincent du Vigneaud and finding that it, too, was highly active.

(3) *Observation that GSH is Released into the Water by Living Animals Only after They Have Been Stung by the Penetrant Nematocysts of Hydra*

The above results indicated that living prey release GSH as a "feeding hormone" into the water. That only injured animals release GSH was shown by placing a living daphnia in a drop of water in a depression slide together with a hydra from which all the tentacles had been removed. When the slide was examined under a dissecting microscope, it was found that the mouth of the hydra did not open as it does in the presence of fresh tissue juice. Adding the severed tentacles to the same drop, however, shortly resulted in the hydra's mouth opening widely, the detached tentacles being observed to sting the daphnia on contact.

As hydra have four types of nematocysts, each with its own specific function (Ewer, 1947), it was of interest to determine which of these types was responsible for the release of tissue juice from living animals.

TABLE 2

RELEASE OF SH COMPOUNDS BY LIVING CRUSTACEA STUNG BY THE NEMATOCYSTS OF HYDRA

Sample of water surrounding:	*Concentration of SH compounds (as GSH)**
	γ/ml.
Hydra alone	0.0
Living crustacea alone	0.2
Dead crustacea alone	0.0
Hydra mixed with living crustacea	4.0
Hydra mixed with dead crustacea	0.0

*Method of Grunert and Phillips, 1951.

Careful microscopic observation (×500) showed that tissue juice leaked out of the holes punctured in daphnia by the long barbed filaments shot out by the penetrant nematocysts (stenoteles). None of the three other kinds of nematocysts had this action on crustacea.

Direct evidence that the tissue juice released in this fashion contained SH groups was obtained by chemical analysis. It was found (TABLE 2) that SH groups appeared in the water surrounding crustacea (*Artemia*) only when two conditions were fulfilled: (a) the crustacea were alive; and (b) they had been stung by the nematocysts of hydra, or otherwise injured.

(4) *Specificity of GSH in the feeding reaction*

In order to determine whether hormonal activity was limited to GSH, various closely related compounds were obtained and tested. Professor Heinrich Waelsch kindly supplied the various dipeptides listed in TABLE 3. Professor Vincent du Vigneaud kindly supplied aspartathione as well as the synthetic GSH mentioned above.

The results obtained in TABLE 3 indicated that hormonal activity was strictly limited to reduced glutathione. Any change in the molecule of GSH resulted in its inactivation. For example, either oxidation or acetylation of the SH group of GSH destroyed its activity. Partial hydrolysis of GSH into its component dipeptides likewise produced only inactive compounds.

Even aspartathione was found to be unable to induce the feeding reaction in hydra, although this aspartic acid analog of GSH resembles GSH so closely that it demonstrates coenzyme activity in the otherwise GSH-specific glyoxalase reaction (Behrens, 1941).

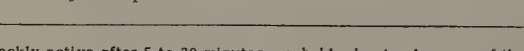
TABLE 3

SPECIFICITY OF GLUTATHIONE IN THE FEEDING REACTION

Substance	*Hormonal activity*
Fresh tissue juice	+
Reduced glutathione-natural	+
Reduced glutathione-synthetic	+
Oxidized glutathione	–
S-acetylglutathione*	–
Aspartathione	–
γ Glutamyl-cysteine	–
Cysteinyl-glycine	–
Glutamic acid	–
Glutamine	–
Cysteine	–
Glycine	–
Coenzyme A (1% Sigma)	–
N-acetyl-cysteine	–
γ Glutamyl-alanine	–
Glycyl-cysteine	–
Na hydrosulphite	–

*Weakly active after 5 to 30 minutes, probably due to cleavage of the S-acetyl bond.

Hormonally active *Glutathione* (γ-glutamyl-cysteinyl-glycine)



Hormonally inactive *Aspartathione* (β-aspartyl-cysteinyl-glycine)

In the table above, 1 mg. of the substance to be tested was dissolved in 1 ml. 0.1 mg. 1 $NaHCO_3$. Dilute NaOH was added when necessary to bring the solution to pH 7.0 using bromthymol blue as an indicator. This solution was then poured into 3 ml. of the same buffer containing 10 to 20 hydra starved 2 to 4 days. A dissecting microscope was used to examine the hydra for the presence or absence of the characteristic "feeding reaction."

This specific response of hydra to GSH is so sensitive that it may be used as a specific bioassay for this compound (Loomis, 1955). Since 0.001 mg. GSH/ml. is sufficient to elicit the reaction, as little as 0.1 per cent GSH may be detected in 1 mg. samples of unknown. None of the amino acids or peptides listed in TABLE 3 were found to interfere with the reaction. The specificity of this hydra assay appears to be superior to the other known assays listed by Patterson and Lazarow (1955).

TABLE 4

EFFECT OF STARVATION ON THE SENSITIVITY OF HYDRA TO GSH

Days since last feeding	*Concentration of glutathione*				
	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M
0	+	+	0	0	0
1	++	++	+	0	0
2	++	++	+	+	0
4	++	++	++	+	0
8	++	++	++	++	+

0: no observable reaction
+: writhing of the tentacles only
++: tentacle contraction and mouth opening

(5) *GSH Induction of the "Feeding Reaction"*

It was found that hydra respond in a graded fashion to increasing concentrations of GSH. The most sensitive response consists of an active writhing of the tentacles, as if in search of food. Higher concentrations of GSH induce the two parts of the standard "feeding reaction": (1) a characteristic posture of the contracted tentacles about the mouth, as illustrated in FIGURE 5; and (2) an opening of the mouth itself as seen in FIGURE 2. These responses are induced by different concentrations of GSH, depending on the degree of starvation of the hydra involved (TABLE 4).

Due to the autoxidizable nature of GSH, the dilute solutions utilized in TABLE 4 were prepared immediately before use by diluting a 10^{-2}M solution of GSH the appropriate number of times. Solutions of GSH as strong as 10^{-2}M are stable if protected from the atmosphere, as all of the air dissolved in them (2.7×10^{-4}M O_2 at 20° C.) becomes gradually reduced, thus rendering them anaerobic.

(6) *Induction of Eversion and Cannibalism by GSH*

In addition to inducing the normal "feeding reaction" in hydra, GSH solutions frequently induced hydra to fasten their lips to the walls of whatever container they were in. Gradually expanding the circles of their mouths, such hydra gradually flattened themselves into pancake-shaped discs markedly different from their usual cylindrical form (FIGURE 3). Usually this reaction proceeded further on surfaces of gelatine or agar than on glass itself, proceeding to completion at times with small specimens. Eventually, the pancake-shaped discs so produced fell off backwards along the body wall, thus partially everting the hydra (FIGURE 4).

Occasional specimens everted completely, so that their ectoderm was internal and their endoderm external, their tentacles now arising from within the body tube and extending out through the opening formed by the inversion of the normal mouth.

In addition to producing eversion as described above, GSH solutions often induced cannibalism in crowded cultures of hydra. The unusual nature of this response is indicated by Trembley's statement that "One polyp never under any circumstances devours another" (quoted by Baker, 1952). FIGURES 5, 6, and 7 illustrate this effect of GSH, showing one hydra swallowing other hydras even when it is being ingested by a third. Occasionally, this reaction also proceeds to completion, one hydra being entirely engulfed by another. Actual digestion of another hydra, however, has never been observed, and usually the ingested hydra is ejected after a few hours.

(7) *Effect of Various Inhibitors on the Feeding Reaction*

(a) *SH inhibitors.* Further evidence that the feeding reactions of hydra are controlled by GSH was obtained by finding that chemical reagents known to react with SH groups completely prevent hydra from feeding on their usual prey. TABLE 5 lists four compounds of this type that produce a typical paralysis of the feeding reaction. It is significant that, in this paralyzed reaction, the hydra are still able to capture prey, but not to swallow them. This demonstrates that these SH reagents do not block the discharge of the nematocysts, but only inactivate GSH as rapidly as it is released into the water by the injured prey, so that the tentacles do not contract towards the mouth but remain extended as illustrated in FIGURE 8.

(b) *Anesthetics.* The various anesthetics listed in TABLE 6 produce an identical paralysis to that produced by SH reagents, but by an entirely different mechanism. Instead of inactivating GSH, these compounds are

TABLE 5

INHIBITION OF THE FEEDING REACTION BY SH REAGENTS

Inhibitor	*Reaction with glutathione*	*Minimal effective concentration*
Hydrogen peroxide	Oxidation of SH to SS	0.1%
Iodoacetate	Addition to SH	10^{-2}M
p-Chloromercuri-benzoate	Mercaptide formation	10^{-4}M
Alloxan	Addition to SH	10^{-2}M

TABLE 6

INHIBITION OF THE FEEDING REACTION BY ANESTHETICS

Anesthetic	*Minimal effective concentration*
Urethane	5×10^{-2}M
Chloretone	3×10^{-3}M
Tricaine methanesulfonate (MS 222 Sandoz)	0.1%

hown to anesthetize nerve cells. These results, therefore, suggested at (1) GSH stimulates the ectodermal sensory cells of hydra resulting (2) the contraction of the longitudinal muscle fibers present in the *djacent* musculoepithelial cells. GSH stimulation apparently does not esult in the contraction of any circular endodermal muscle fibers, for oth (1) tentacle contraction and (2) mouth opening involve only the ontraction of the longitudinal fibers present in the ectoderm. Similarly, SH stimulation does not induce a generalized nervous reflex, for these wo responses occur separately during normal feeding. Mouth opening ccurs only after tentacle contraction has brought living prey into the mmediate vicinity of the mouth itself.

(c) *Partial inhibition of normal feeding by GSH.* When a hydra captures a daphnia, a concentration gradient of GSH gradually forms about the daphnia in the water. This concentration gradient is used by he hydra to locate the prey with its mouth, for the hydra bends towards he side where the concentration of GSH is highest. That such coordiation results from a passive contraction of the muscle fibers on the side where the GSH concentration is highest, and not from any central controlling mechanism, is shown by the following experiment. GSH was added to the water in which hydra were feeding on daphnia, and it was found that such addition seriously interfered with their ability to feed. The reason for this interference is that hydra locate their prey by responding to the *direction* from which the GSH originates. Under normal conditions, all of the GSH present in the water emanates from the prey. When additional GSH is distributed evenly throughout the solution, and in no relation whatsoever to the location of the prey, the hydra become "confused" and their coordination during feeding is impaired.

(8) *Influence of the GSH Mechanism on the Diet of Hydra*

The results obtained in these experiments indicate that hydra will swallow all living animals capable of releasing sufficient GSH into the water about them to activate the "feeding reaction." Although all living

animals are known to contain GSH as a cell constituent (Barron, 1951), they apparently vary quantitatively in the amount of GSH they release following injury by nematocysts. Ewer, for example (1947), has shown that, even though hydra discharge nematocysts into other hydra, they do not then proceed to swallow them. This avoidance of cannibalism (except in the presence of artificially added GSH) clearly indicates that the discharge of nematocysts into some animals results in the release of more GSH than in others.

Investigation of the reason for these differences was approached by determining which of a wide range of animal species would be ingested by hydra and which would not. In each instance, nematocyst discharge was ensured by agitating the test animal among the tentacles of hydra, as had been done previously with dead daphnia.

The results of these experiments are listed in TABLE 7. It was found that hydra would ingest all Nemathelminthes and higher types of animals, but would not ingest specimens of the phyla below the Nemathelminthes with the sole exception of the giant amoeba, *Chaos chaos*.

Since all of the species ingested by hydra (except *Chaos chaos*) possess vascular or other types of intercellular fluids, it was concluded that such animals release GSH when these fluids escape from the animal following puncture by the nematocysts of hydra. Animals without such free intercellular fluids apparently do not release sufficient GSH to activate the "feeding reaction." Such animals may be captured but not swallowed by hydra.

This conclusion was confirmed in two ways. TABLE 2 demonstrates that densely packed hydra do not release detectable amounts of GSH into the water surrounding them, even though they discharge large quantities of nematocysts into one another under these conditions. Secondly, the giant amoeba *Chaos chaos* provides the exception that proves the rule, for such large amounts of *intra*cellular fluid escape on rupture of its cell membrane that threshold quantities of GSH appear in the surrounding water.

From these and related experiments, it was concluded that hydra are specific in their diet in several respects. They require living prey, for example, that are small enough to be captured and yet large enough and active enough to discharge penetrant nematocysts on impact. In addition, hydra require prey that contain vascular, coelomic, or pseudocoelomic fluids enclosed within a thin enough body wall so that nematocyst puncture results in the release of sufficient quantities of GSH to activate the "feeding reaction." As all animals lower than the Nemathelminthes do not contain such fluids, it was concluded that the avoidance of cannibalism by hydra is a general rather than a specific phenomenon.

TABLE 7

SPECIES SPECIFICITY OF THE PREY INGESTED BY HYDRA

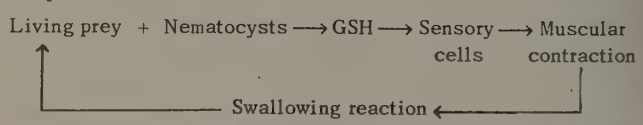
*Species**	*Presence of pseudocoelomic, coelomic, or vascular fluids*	*Swallowed by hydra if captured by tentacles*
Protozoa		
Paramecium	–	– (Ewer)
Chaos chaos	–	+
Porifera		
Spongilla	–	–
Coelenterata		
Hydra vulgaris	–	– (Ewer)
Hydra littoralis	–	–
Chlorohydra viridissima	–	–
Platyhelminthes		
Planaria maculata	–	–
Stenostomum	–	–
Polycelis	–	– (Ewer)
"Small rhabdocoel"	–	– (Ewer)
Trochelminthes		
Philodina	–	–
Monostyla	–	–
Nemathelminthes		
Anguillula	+	+
Rhabditis	+	+
Cephalobus	+	+
Chiloplacus	+	+
Annelida		
Tubifex	+	+
"	+	+ (Ewer)
Enchytraeus	+	+
Stylaria	+	+
Nais	+	+
Mollusca		
Limnea stagnalis	+	+ (Ewer)
Arthropoda		
Daphnia	+	+
Cyclops	+	+
Artemia	+	+
Alonella	+	+
Bosmina	+	+ (Ewer)
"Copepoda"	+	+ (Ewer)
Chordata		
Tadpoles	+	+ (Trembley)
"Piece of tadpole tail"	+	+ (Ewer)
Newly-hatched fish	+	+ (Trembley)

*All species obtained from Carolina Biological Supply Co., Elon College, North Carolina.

Discussion

Modern authors disagree on the role of the nervous system in hydra. Hyman (1940) states that there is little evidence for a central controlling mechanism, and that their nervous system is characterized by an "extreme autonomy or independence of parts." In contrast to this, Buchsbaum (1948) states that the nerve net of hydra "coordinates the muscular contractions involved in swallowing food." For example, the mouth "opens in 'anticipation' before the food has actually touched the sensory cells around its edge." The nerve net of hydra therefore "enables an animal composed of many thousands of cells to react as one integrated individual."

The hormonal mechanism described in this paper explains how an animal as primitive as hydra, with a marked "autonomy or independence of parts" can still react as "one integrated individual." It shows that the integrated character of the feeding reaction results from a sensitive feed-back mechanism operating hormonally between the hydra and its prey. The cyclic reaction mediated by GSH can be represented diagrammatically as follows:



The effectiveness of this mechanism is largely due to the sluggish character of glutathione autoxidation, for GSH can survive in solution long enough to coordinate the swallowing process and yet be eventually removed through oxidation to the disulfide. Furthermore, the presence of GSH in the water surrounding hydra is an effective indicator of the presence of nutritious particles attached to the tentacles in the form of living prey, for GSH is not found in nature apart from living cells.

The evolutionary significance of the GSH mechanism is interesting to consider. Although this mechanism might be considered a first step towards the development of a sense of smell, it is more likely that it represents the first beginnings in the development of the endocrine systems found in higher animals. Thus, it is significant that living prey do not emit GSH under normal conditions, but only after having been physically captured by hydra. The GSH mechanism, therefore, does not aid hydra in obtaining food. It functions in the immediate vicinity of the mouth and tentacles to coordinate the ingestion of prey that have already been captured. The GSH mechanism moreover, resembles the endocrine systems of higher animals in that it is chemically specific.

Table 7 demonstrates that hydra feed exclusively on animals that evolved many millions of years after the appearance of the first coelenter-

s. Hydra are not, therefore, to be regarded as simple ancestral coelen-
tes still surviving as "living fossils." They represent the final
ılt, rather, of a secondary adaptation that took place millions of years
er, after evolution had progressed to a point where the worms and
stacea on which hydra currently feed had already appeared upon this
th. This conclusion confirms the view of modern authors who con-
de, from morphological evidence, that hydra are offshoots "which
ough adaptation to fresh-water life have lost all trace of a medusan
m" (Hyman, 1940).

It has been found that the GSH mechanism described in this paper,
ng *Hydra littoralis*, is also present in the green hydra *Chlorohydra*
idissima, although in a less sensitive form. Only further investigation
n define the generality of this mechanism throughout the coelenterates.
this connection, Beutler (1926) and Hyman (1940) have observed that
ny different hydroids and hydromedusae feed exclusively on living
imals and yet will ingest nonliving particles that have been soaked in
esh tissue juice. Obe (1938), likewise, has found that the mouths and
ntacles of *Fungia* and other corals respond to the coelomic fluids of
ollusks, even when such fluids have been diluted as much as 2,500
mes.

From these and other observations, it seems possible that all
oelenterates possess hormonally-controlled "feeding reactions" that
unction in conjunction with the penetrant nematocysts that are charac-
eristic of the phylum Cnidaria. The chemical mediator involved may
onsist of glutathione in certain cases as in hydra, or may consist of
ome other cell constituent that functions in a similar manner.

I should like to express my appreciation for the conscientious assis-
ance of John H. Kirhoffer throughout this problem and to Doctor Howard
M. Lenhoff* for his aid and advice in the preparation of the manuscript.

Summary

(1) After hydra have captured living prey with their tentacles, they demonstrate a coordinated "feeding reaction."
(2) This "feeding reaction" has been found to be controlled chemically by a "feeding hormone" and not by the neuromuscular system.
(3) The "feeding hormone" has been specifically identified as reduced glutathione (GSH).
(4) Hydra feed exclusively on living prey, as only living animals release GSH following injury by the nematocysts of hydra.
(5) Hydra feed exclusively on living specimens of the Nemathelminthes

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and higher phyla. Only these species possess the vascular, coelomic, and pseudocoelomic fluids that can flow out into the water following injury by the penetrant nematocysts of hydra, thus releasing GSH into the water about them.

(6) This restriction in the diet of hydra indicates that hydra today are the final result of a secondary adaptation that took place only after higher forms of life, such as Nemathelminthes and the higher phyla, had appeared on earth.

(7) Hydra do not feed on other hydra (cannibalism) or, in fact, on any species below the Nemathelminthes, as these animals do not contain such free-flowing intercellular fluids.

(8) Cannibalism among hydra may be artificially induced by suspending hydra in solutions of synthetic GSH.

(9) Solutions of GSH frequently induce hydra to attempt to swallow the glass walls of their container. During such attempts, hydra often expand their mouths to such an extent that they either completely or partially invert themselves.

(10) The GSH reaction is completely specific. No other compounds can substitute for it, including oxidized glutathione, cysteine, γ glutamyl-cysteine, cysteinyl-glycine and β-aspartyl-cysteinyl-glycine.

(11) The "feeding reaction" is sensitive to 10^{-6}M GSH and may be used as a specific bioassay for as little as 1 γ of this compound.

(12) The ability of hydra to feed on living prey is inhibited by dilute solutions of alloxan, iodoacetate, p-chloromercuri-benzoate, and hydrogen peroxide. These chemicals are known to react with GSH and other SH compounds.

(13) It is concluded that the integrated feeding reactions of *Hydra littoralis* are initiated and controlled by reduced glutathione.

(14) The phylogenetic significance of this hormonal mechanism is discussed.

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